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·研究报告·

AtMYB77促进NO合成参与调控干旱胁迫下拟南芥侧根发育

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摘要 转录因子MYB77与信号分子一氧化碳(NO)是侧根发育的重要调节因子, 但MYB77和NO在干旱胁迫下侧根发生中的作用及机制尚不明确。该文以拟南芥(*Arabidopsis thaliana*)野生型、*AtMYB77*缺失突变体*Atmyb77-1*及过表达株系AtOE77-1和AtOE77-3为材料, 研究了MYB77和NO在干旱胁迫下侧根发生中的作用。结果表明, *AtMYB77*受干旱胁迫诱导, *AtMYB77*缺失导致干旱胁迫下侧根发育相关基因*CYCA2;1*和*CDKA;1*表达下调, 同时*Atmyb77-1*的侧根数目和长度显著低于野生型, *AtMYB77*过表达则作用相反, 表明AtMYB77参与干旱胁迫下侧根发育的调控过程。干旱胁迫下, 拟南芥根系NO含量显著升高, NO合成关键酶NO合酶(NOS)和硝酸还原酶(NR)活性及基因表达上调, *Atmyb77-1*中NO含量、NOS和NR活性及基因表达量显著低于野生型, 而AtOE77-1和AtOE77-3根系NO含量及合成酶活性和基因表达量显著高于野生型。外施NO供体硝普钠(SNP)能缓解*AtMYB77*缺失对*CYCA2;1*和*CDKA;1*表达及侧根生长的抑制, NO清除剂或合成抑制剂则削弱*AtMYB77*过表达对侧根生长的促进作用。上述结果表明, AtMYB77通过促进NO合成参与干旱诱导的拟南芥侧根生长过程, 研究结果为深入解析干旱诱导侧根生长的信号转导机制和培育耐旱植物奠定了理论基础。

关键词 AtMYB77, NO, 侧根发育, 干旱胁迫, 拟南芥

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干旱是世界范围内严重威胁农业生产的主要非生物胁迫, 全球约一半以上的农作物产量损失由干旱引起。根是植物吸收水分的主要器官, 干旱胁迫下, 维持根系的正常生长发育和功能对植物体水分代谢平衡至关重要(Chakhchar et al., 2018; Zhou et al., 2018; Bashir et al., 2019)。侧根是植物根系的主要组成部分, 其发育受内部信号及土壤水分和养分状况等环境信号的调控。多种内外因素通过内源激素信号介导的转录调控影响侧根发育相关基因的表达, 进而调控侧根发育。研究表明, MYB、AP2/ERF和bZIP等转录因子家族的许多成员参与侧根发育相关基因表达的调控(Dash et al., 2017; Gu et al., 2017; Nie et al., 2018)。其中, MYB是植物特有的转录因子家族, 广泛参与植物生长发育和逆境适应等过程的调节(车永梅等, 2018; An et al., 2020; 张雨等, 2020)。该家族的多个成员与侧根发育的调控相关, 但作用各不相同。例如, 拟南芥(*Arabidopsis thaliana*) *AtMYB61*缺失后表现出侧根形成受阻; 水稻(*Oryza sativa*) R2R3-类型MYB转录因子*OsMYB1*缺失抑制赤霉素诱导的侧根生长, 是侧根发育的正调节因子(Romano et al., 2012; Gu et al., 2017); AtMYB96和AtMYB93则在侧根发育过程中起负调控作用, 其缺失后促进拟南芥的侧根发育, 使侧根密度增大(Seo and Park, 2009; Gibbs et al., 2014); 毛果杨(*Populus trichocarpa*) R2R3类MYB转录因子*PtrSSR1*基因受盐胁迫诱导, 参与盐胁迫下侧根发生过程的调节(Fang et al., 2017)。有研究表明, MYB77在脱落酸(abscisic acid, ABA)介导的侧根发育过程中起重要作用, 在ABA作

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用后期的侧根恢复生长阶段, ABA受体PLY8和PLY9与MYB77结合, 诱导多个生长素响应基因的表达, 进而促进拟南芥侧根的生长(Zhao et al., 2014; Xing et al., 2016)。ABA是重要的水分胁迫信号, MYB77是否参与干旱胁迫下侧根发育的调控及其作用机理目前尚未见报道。

一氧化氮(nitric oxide, NO)是植物体内广泛存在的气体信号分子(张玲玲等, 2017)。植物体内NO的合成途径包括酶促和非酶促途径, 其中硝酸还原酶(nitrate reductase, NR)和一氧化氮合酶(nitric oxide synthase, NOS)是合成NO的关键酶。拟南芥中, NR由*AtNia1*和*AtNia2*两个基因编码, 目前已鉴定的拟南芥NOS基因为*AtNOA1* (Xie et al., 2013)。有研究表明, NO参与介导植物体内水分胁迫的信号转导过程, 通过影响渗透调节物质的积累、抗氧化酶活性和气孔运动等增强植物的抗旱性(Santisree et al., 2015; Wang et al., 2015; Cao et al., 2019; Sahay et al., 2019)。NO亦参与调控植物侧根发育过程, 其能够诱导细胞周期调控基因*CYCA2;1*、*CYCA3;1*、*CYCD3;1*、*CDKA1*和*KRP2*等的表达, 进而促进侧根的形成(Correa-Aragunde et al., 2004, 2006)。NO是否参与干旱胁迫下侧根的发育? NO与MYB77之间是否存在互作? 作用机制是什么? 目前尚未见报道。本研究以*AtMYB77*缺失突变体及过表达株系为实验材料, 研究MYB77与NO在干旱胁迫下侧根发育中的互作及机制, 可为深入解析干旱诱导侧根发育的信号转导机理奠定基础。

1 材料与方法

1.1 实验材料

拟南芥(*Arabidopsis thaliana* L.)野生型(Col-0生态型)购自美国拟南芥生物资源中心, 拟南芥*AtMYB77* T-DNA插入突变体(*SALK_067655*, 命名为*Atmyb77-1*)由朱健康老师(中国科学院上海植物逆境生物学研究中心)惠赠, *AtMYB77*过表达植株AtOE77-1和AtOE77-3由本实验室构建。

NO荧光探针DAF-FM DA购于碧云天生物技术公司(上海)。NO供体硝普钠(sodium nitroprusside, SNP)、NO专一性清除剂(carboxy-PTIO potassium salt solid, c-PTIO)、NO合成抑制剂(NG-nitro-L-arginine methyl ester hydrochloride, L-NAME)和钨酸钠(sodium tungstate dihydrate, Na_2WO_4)均购自Sigma公司(美国)。其它试剂为国产分析纯。

1.2 实验材料培养和处理

将野生型拟南芥、*AtMYB77*缺失突变体及过表达植株种子用10% NaClO消毒15分钟, 无菌水清洗数次, 然后接种于MS固体培养基上。4°C黑暗条件下培养2–4天, 然后转入光照培养箱垂直生长4天。挑选长势一致的幼苗于MS营养液中、含60 mmol·L^{-1}甘露醇的MS营养液或分别含有0.05 mmol·L^{-1} c-PTIO、0.1 mmol·L^{-1} Na_2WO_4、0. 05 mmol·L^{-1} L-NAME和0.01 mmol·L^{-1} SNP的含60 mmol·L^{-1}甘露醇的MS营养液中, 梯度处理0、15、30、60、120和180分钟后测定*AtMYB77*的表达量; 处理4–6小时后测定*AtNOA1*、*AtNia1*和*AtNia2*的表达量及NOS和NR活性与NO含量; 处理12小时后测定*CYCA2;1*和*CDKA;1*的表达量; 处理14天后观察并测定拟南芥根系的生长状况, 统计侧根数目和长度。

1.3 NO的荧光检测

参考刘国华等(2009)的方法并稍作修改。在20 μmol·L^{-1} HEPES-NaOH buffer (pH7.4)中加入5 μmol·L^{-1} DAF-FM DA, 使其终浓度为1 μmol·L^{-1}。将1周龄生长状态良好的幼苗根放入溶液中, 黑暗孵育20分钟后, 用缓冲液冲洗3次, 以确保染料完全清洗干净。最后将根放置于载玻片上, 盖好盖玻片, 用488 nm蓝色光激发, 发射波长为515 nm, 经激光共聚焦扫描显微镜(LEICA TCS SP5 II)扫描。

1.4 NO含量和NOS活性测定

用NO和NOS试剂盒(南京建成生物工程研究所, 中国)测定NO和NOS活性。

1.5 NR活性测定

NR活性测定参照刘国华等(2009)的方法。

1.6 基因表达量检测

利用实时荧光定量PCR技术检测基因的表达量。用CTAB法提取总RNA, 在NCBI数据库中检索基因序列, 用primer blast设计引物, 所用引物见表1。以

表1 定量PCR引物序列

Table 1 The primers used for quantitative PCR analysis

Primer name	Primer sequence (5′–3′)
AtMYB77-FP	GGAGAAGGACGTAGAGGTGAG
AtMYB77-RP	GGTGTTATTACTCCACAATCCCTA
AtCDKA;1-FP	GAGGATACATGGCGTGGGGTA
AtCDKA;1-RP	GCGTTGATTCTTTTGGTCGGA
AtCYCA2;1-FP	GCCCCTGAAATCCACTACAAT
AtCYCA2;1-RP	AGAGACCTCCACAAGCCAATC
AtNia1-FP	AGGTCCACTAGGGCACATCG
AtNia1-RP	TTCGTCCTCTGGATCACTCAATAT
AtNia2-FP	TTCTTACAAACCTCCCGTTCCAG
AtNia2-RP	GATTTTCTTATCATCTCCTTGTAGT
AtNOS1-FP	GATTCTCCGGGATTTGTCGA
AtNOS1-RP	CCTCCATTACCACCAACTGCT

PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio)反转录获得的cDNA为模板。实时荧光定量PCR程序为: 94°C5分钟; 94°C30秒, 57°C30秒, 72°C30秒, 30个循环; 每个样品重复3次。以*Actin*为内参基因, 用$2^{-\Delta\Delta CT}$方法(Willems et al., 2008)计算基因的相对表达量。

1.7 数据统计方法

每个处理取6棵植株, 分别测量侧根长度和数目, 其它实验结果均为3次重复的平均值±标准误。采用DPS软件对数据进行显著性分析; 用Duncan新复极差法进行多重比较。

2 结果与讨论

2.1 *AtMYB77*受干旱胁迫诱导表达

用60 mmol·L^{-1}甘露醇模拟干旱胁迫, 检测干旱胁迫对*AtMYB77*表达的影响。结果显示, 干旱胁迫诱导拟南芥根中*AtMYB77*表达量升高, 于处理15分钟时达到峰值, 随后降低(图1)。

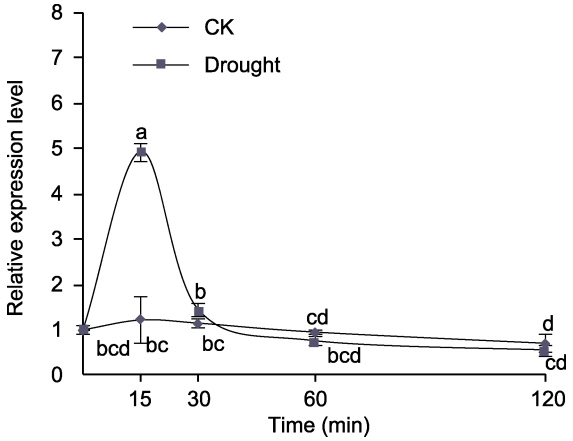

图1 干旱胁迫对拟南芥根部*AtMYB77*表达的影响

CK: 对照。不同小写字母表示不同处理间差异显著(P<0.05)。

Figure 1 The effect of drought stress on *AtMYB77* expression in *Arabidopsis* roots

CK: Control. Different lowercase letters indicate significant differences among different treatments at P<0.05.

2.2 干旱胁迫下*AtMYB77*参与调控拟南芥侧根的发育过程

*AtMYB77*受干旱胁迫诱导(图1), MYB77在侧根发育过程中起重要作用(Zhao et al., 2014; Xing et al., 2016)。MYB77是否参与干旱胁迫下侧根发育的调控? 我们检测了干旱胁迫对*AtMYB77*缺失突变体及过表达株系侧根数目及长度的影响。结果显示, 正常条件下*AtMYB77*缺失或过表达对侧根生长无显著影响; 干旱诱导拟南芥侧根数目增加, 且*AtMYB77*过表达植株侧根数目以及长度均显著高于野生型拟南芥(P<0.05), 缺失突变体*Atmyb77-1*则显著低于野生型(P<0.05) (图2A–C), 表明*AtMYB77*参与干旱胁迫下侧根发生的调控。

正常条件下, *AtMYB77*过表达植株中侧根发育相关基因*AtCYCA2;1*和*AtCDKA;1*的表达量明显高于野生型和*AtMYB77*缺失突变体; 干旱胁迫12小时, 野生型拟南芥和过表达植株中*AtCYCA2;1*和*AtCDKA;1*的表达量显著上升, 并且过表达植株中上升幅度更大, 而缺失突变体*Atmyb77-1*中*AtCYCA2;1*以及*AtCDKA;1*的表达量上调受到抑制, 但差异不显著(图2D, E)。推测*AtMYB77*可能通过调控*AtCYCA2;1*和*AtCDKA;1*或其它侧根发育相关基因的表达参与调控侧根的发生, 进而应答干旱胁迫。

2.3 干旱胁迫下*AtMYB77*调控拟南芥根部NO合成

NO是植物体内广泛存在的气体信号分子, 能够诱导细胞周期调控基因的表达, 进而促进侧根的形成(Correa-Aragunde et al., 2006)。我们利用激光共聚焦显微技术和定量测定方法研究了干旱胁迫下拟

南芥及*AtMYB77*缺失或过表达株系根部NO含量的变化。结果表明，正常条件下，*AtMYB77*过表达植株和野生型植株根中NO含量显著高于缺失突变体(P<0.05)；干旱条件下，野生型拟南芥和过表达株系根中NO含量显著增加(P<0.05)，过表达植株中增加幅度大于野生型，缺失突变体*Atmyb77-1*根中NO含量无显著变化(图3A, B)。表明*AtMYB77*可调控NO的合成，参与干旱胁迫的应答过程。

植物体内NO的酶促合成途径主要包括NOS和NR途径，我们检测了干旱胁迫下拟南芥根中NOS和NR活性及相关基因的表达量变化。结果显示，干旱胁迫下，野生型拟南芥和*AtMYB77*过表达植株NOS活性增强，*AtNOS1*和*AtNia2*表达量显著增加(P<0.05)，*AtMYB77*过表达植株*AtNia1*的表达量也显著增加(P<0.05)，而缺失突变体*Atmyb77-1*中干旱诱导的NO合成酶活性和相关基因表达上调受到抑制(图3C–G)。推测干旱胁迫下*AtMYB77*通过NR和NOS途径调控NO合成，且NOS途径为主要调控途径。

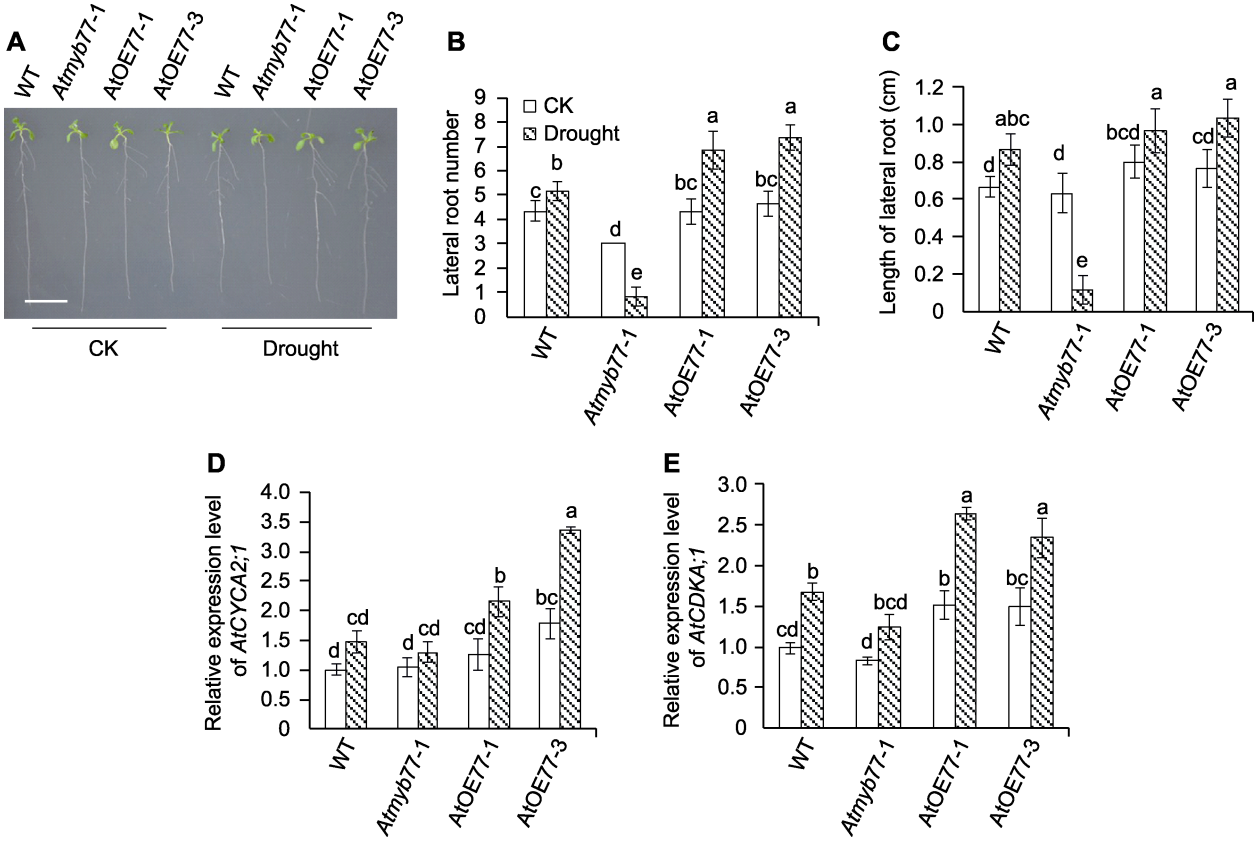

图2 干旱对拟南芥*Atmyb77-1*突变体和*AtMYB77*过表达株系侧根生长和发育相关基因表达的影响

(A) 干旱胁迫下*Atmyb77-1*突变体根系生长表型(Bar=1 cm)；**(B)** 干旱对拟南芥*Atmyb77-1*突变体和*AtMYB77*过表达株系侧根数目的影响；**(C)** 干旱对拟南芥*Atmyb77-1*突变体和*AtMYB77*过表达株系侧根长度的影响；**(D)** 干旱对拟南芥*Atmyb77-1*突变体和*AtMYB77*过表达株系根部*AtCYCA2;1*表达的影响；**(E)** 干旱对拟南芥*Atmyb77-1*突变体和*AtMYB77*过表达株系根部*AtCDKA;1*表达的影响。CK: 对照; WT: 野生型。不同小写字母表示不同株系的不同处理间差异显著(P<0.05)。

Figure 2 Effects of drought stress on lateral root growth and expression of lateral root development related genes in *Arabidopsis Atmyb77-1* mutant and *AtMYB77* overexpression lines

(A) Root phenotypes of *Atmyb77-1* mutant under drought stress (Bar=1 cm); **(B)** Effects of drought stress on lateral root number of *Atmyb77-1* mutant and *AtMYB77* overexpression lines; **(C)** Effects of drought stress on lateral root length of *Atmyb77-1* mutant and *AtMYB77* overexpression lines; **(D)** Effects of drought stress on relative expression level of *AtCYCA2;1* in roots of *Arabidopsis Atmyb77-1* mutant and *AtMYB77* overexpression lines; **(E)** Effects of drought stress on relative expression level of *AtCDKA;1* in roots of *Arabidopsis Atmyb77-1* mutant and *AtMYB77* overexpression lines. CK: Control; WT: Wild type. Different lowercase letters indicate significant differences among different treatments of different lines at P<0.05.

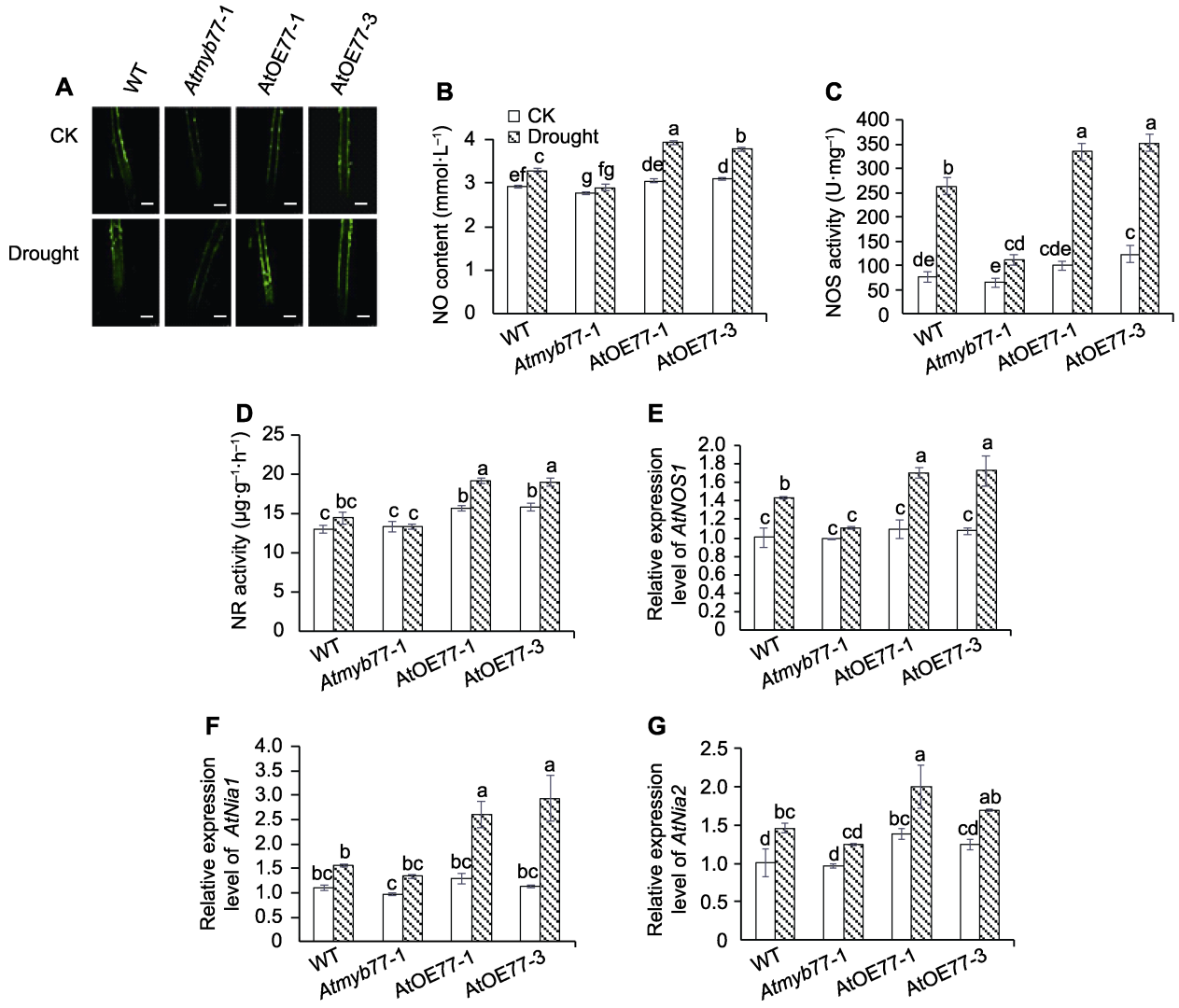

图3 干旱对拟南芥根部NO含量及NO合成酶活性和相关基因表达的影响

(A) 干旱胁迫下拟南芥根部NO荧光成像(Bars=100 μm); **(B)** 干旱对拟南芥根部NO含量的影响; **(C)** 干旱对拟南芥根部NOS活性的影响; **(D)** 干旱对拟南芥根部NR活性的影响; **(E)** 干旱对拟南芥根部*AtNOS1*表达量的影响; **(F)** 干旱对拟南芥根部*AtNia1*表达量的影响; **(G)** 干旱对拟南芥根部*AtNia2*表达量的影响。CK: 对照; WT: 野生型; NOS: 一氧化氮合酶; NR: 硝酸还原酶。不同小写字母表示不同株系的不同处理间差异显著(P<0.05)。

Figure 3 Effects of drought stress on NO content, activities and gene expression of NO synthesis enzymes in *Arabidopsis* roots

(A) NO fluorescence imaging of *Arabidopsis* roots under drought stress (Bars=100 μm); **(B)** Effects of drought stress on NO content in *Arabidopsis* roots; **(C)** Effects of drought stress on NOS activity in *Arabidopsis* roots; **(D)** Effects of drought stress on NR activity in *Arabidopsis* roots; **(E)** Effects of drought stress on relative expression level of *AtNOS1* in *Arabidopsis* roots; **(F)** Effects of drought stress on relative expression level of *AtNia1* in *Arabidopsis* roots; **(G)** Effects of drought stress on relative expression level of *AtNia2* in *Arabidopsis* roots. CK: Control; WT: Wild type; NOS: Nitric oxide synthase; NR: Nitrate reductase. Different lowercase letters indicate significant differences among different treatments of different lines at P<0.05.

2.4 外源NO缓解*AtMYB77*缺失对侧根发育的抑制作用

为探究干旱胁迫下*AtMYB77*位于NO的上游参与调控侧根生长过程的可能性，对干旱胁迫下的*AtMYB77*缺失突变体进行外施NO供体SNP处理。结果表明，外施NO供体可缓解*AtMYB77*缺失对侧根形成(图4A–C)及*AtCYCA2;1*和*AtCDKA;1*表达的抑制作用(图4D, E)。

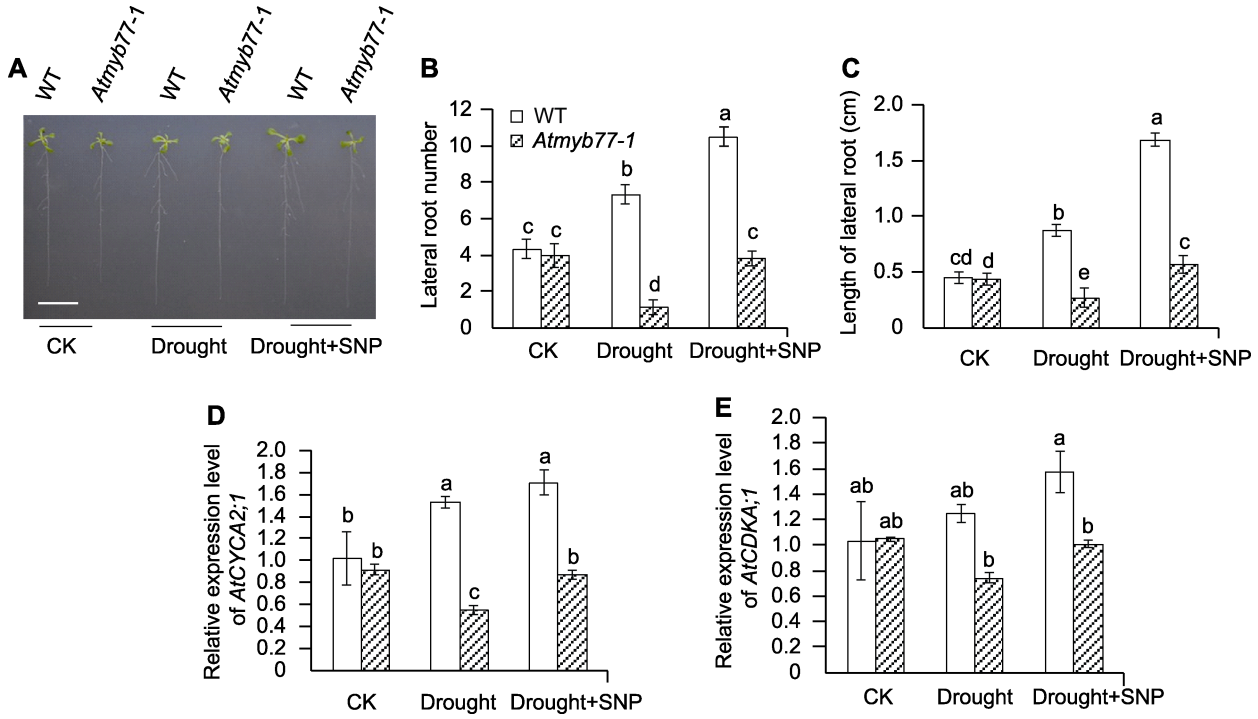

图4 NO供体硝普钠(SNP)对干旱条件下拟南芥*Atmyb77-1*缺失突变体侧根生长和发育关键基因表达的影响

(A) SNP对干旱胁迫下*Atmyb77-1*突变体根系生长的影响(Bar=1 cm); **(B)** SNP对干旱条件下*Atmyb77-1*缺失突变体侧根数目的影响; **(C)** SNP对干旱条件下*Atmyb77-1*缺失突变体侧根长度的影响; **(D)** SNP对干旱条件下*Atmyb77-1*缺失突变体*AtCYCA2;1*表达的影响; **(E)** SNP对干旱条件下*Atmyb77-1*缺失突变体*AtCDKA;1*表达的影响。CK: 对照; WT: 野生型。不同小写字母表示不同株系的不同处理间差异显著(*P*<0.05)。

Figure 4 Effects of NO donor sodium nitroprusside (SNP) on lateral root growth and expression of lateral root development related genes in *Arabidopsis Atmyb77-1* mutant under drought condition

(A) The effect of SNP on root growth of *Atmyb77-1* mutant under drought stress (Bar=1 cm); **(B)** The effect of SNP on lateral root number in *Atmyb77-1* mutant under drought condition; **(C)** The effect of SNP on lateral root length in *Atmyb77-1* mutant under drought condition; **(D)** The effect of SNP on relative expression level of *AtCYCA2;1* in *Atmyb77-1* mutant root under drought condition; **(E)** The effect of SNP on relative expression level of *AtCDKA;1* in *Atmyb77-1* mutant root under drought condition. CK: Control; WT: Wild type. Different lowercase letters indicate significant differences among different treatments of different lines at *P*<0.05.

2.5 NO清除剂和合成抑制剂削弱*AtMYB77*过表达对干旱胁迫下侧根发育的促进作用

为进一步明确干旱胁迫调控侧根生长过程中NO与*AtMYB77*的关系，我们检测了NO清除剂或抑制剂对干旱胁迫下*AtMYB77*过表达植株侧根数目和长度的影响。结果显示，外施NO清除剂(c-PTIO)或抑制剂(L-NAME和Na_2WO_4)后，*AtMYB77*过表达和野生型拟南芥植株侧根数目和长度的增长均受到抑制(图5A–C)；进而利用荧光定量PCR技术检测外施c-PTIO、L-NAME和Na_2WO_4后野生型拟南芥与*AtMYB77*过表达株系侧根相关基因*AtCYCA2;1*和*AtCDKA;1*的表达量变化。结果显示，干旱胁迫下，外施NO清除剂和合成抑制剂后，野生型拟南芥及*AtMYB77*过表达株系中*AtCYCA2;1*和*AtCDKA;1*的表达量显著降低(图5D, E)。上述结果表明，干旱胁迫下*AtMYB77*通过促进NO合成调节侧根的生长发育。

2.6 讨论

侧根是植物根系的主要组成部分，侧根的发育状况与植物的抗旱性关系密切。已有研究表明，生长素是调控侧根发育的主要植物激素，参与调控侧根形成过程的每个阶段(Fukaki et al., 2007); ABA、NO、乙烯和赤霉素等均参与侧根发育的调控(Correa-Aragunde et al., 2006; Zhao et al., 2014; Xing et al., 2016; Hu

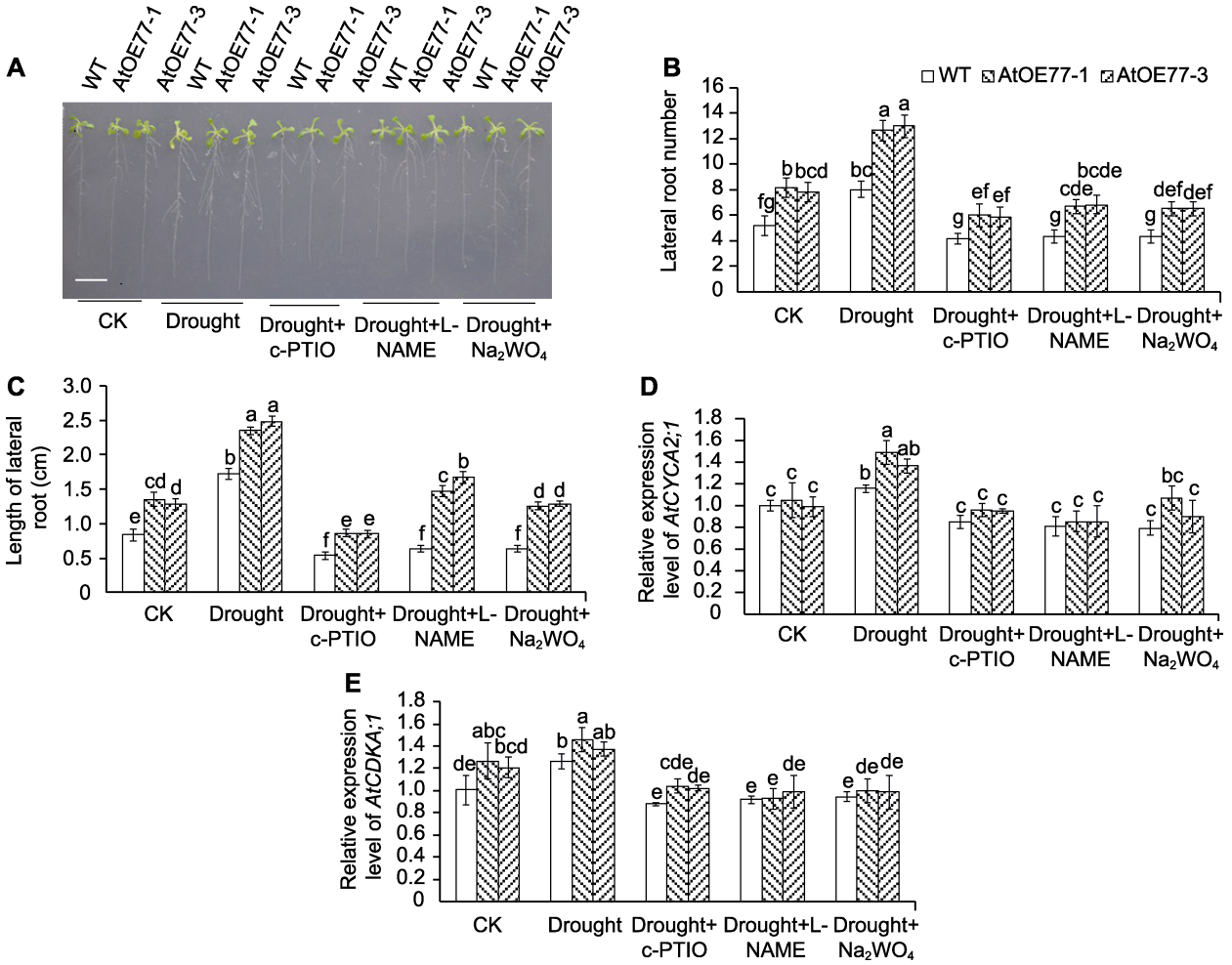

图5 NO清除剂(c-PTIO)或合成抑制剂(L-NAME)对干旱条件下*AtMYB77*过表达株系侧根生长和发育关键基因表达的影响
(A) NO清除剂或合成抑制剂对干旱胁迫下*AtMYB77*过表达株系根系生长的影响(Bar=1 cm); **(B)** NO清除剂或合成抑制剂对干旱条件下*AtMYB77*过表达株系侧根数目的影响; **(C)** NO清除剂或合成抑制剂对干旱条件下*AtMYB77*过表达株系侧根长度的影响; **(D)** NO清除剂或合成抑制剂对干旱条件下*AtMYB77*过表达株系根部*AtCYCA2;1*表达量的影响; **(E)** NO清除剂或合成抑制剂对干旱条件下*AtMYB77*过表达株系根部*AtCDKA;1*表达量的影响。CK: 对照; WT: 野生型。不同小写字母表示不同株系的不同处理间差异显著(P<0.05)。

Figure 5 Effects of NO scavenger (c-PTIO) or biosynthesis inhibitor (L-NAME) on lateral root growth and expression of lateral root development related genes in *AtMYB77* overexpression lines under drought condition
(A) The effects of NO scavenger or biosynthesis inhibitor on root growth of *AtMYB77* overexpression lines subjected to drought stress (Bar=1 cm); **(B)** The effects of NO scavenger or biosynthesis inhibitor on lateral root number of *AtMYB77* overexpression lines under drought condition; **(C)** The effects of NO scavenger or biosynthesis inhibitor on lateral root length of *AtMYB77* overexpression lines under drought condition; **(D)** The effects of NO scavenger or biosynthesis inhibitor on relative expression level of *AtCYCA2;1* in *AtMYB77* overexpression lines under drought condition; **(E)** The effects of NO scavenger or biosynthesis inhibitor on relative expression level of *AtCDKA;1* in *AtMYB77* overexpression lines under drought condition. CK: Control; WT: Wild type. Different lowercase letters indicate significant differences among different treatments of different lines at P<0.05.

et al., 2018)。转录因子MYB77参与生长素和ABA介导的侧根发育过程(Zhao et al., 2014; Xing et al., 2016); NO通过IAA依赖途径参与调控番茄(*Lycopersicon esculentum*)的侧根发生(Correa-Aragunde et al., 2004), 但MYB77和NO在根系响应干旱胁迫中的作用及机制尚未见报道。本研究利用拟南芥*AtMYB77*缺失和过表达株系为实验材料的研究表明, *AtMYB77*受干旱诱导, *AtMYB77*缺失和过表达株系分别表现出侧根发育受到抑制和促进的表型(图2A–C), 证明AtMYB77介导了干旱调控的侧根发育过程。NOS和

NR是植物体内催化NO合成的主要酶，干旱诱导拟南芥NO的含量升高，NR和NOS活性及基因表达量上调(图3A–E); NOS抑制剂L-NAME和NR抑制剂Na_2WO_4能减弱干旱对*AtMYB77*过表达对侧根发育的促进作用，而NO供体SNP能缓解*AtMYB77*缺失对侧根发育的抑制作用(图4，图5)。上述结果表明，在植物响应干旱的信号转导过程中，NO位于MYB77的下游，MYB77通过NOS和NR途径促进NO合成进而调控干旱诱导的侧根发育。

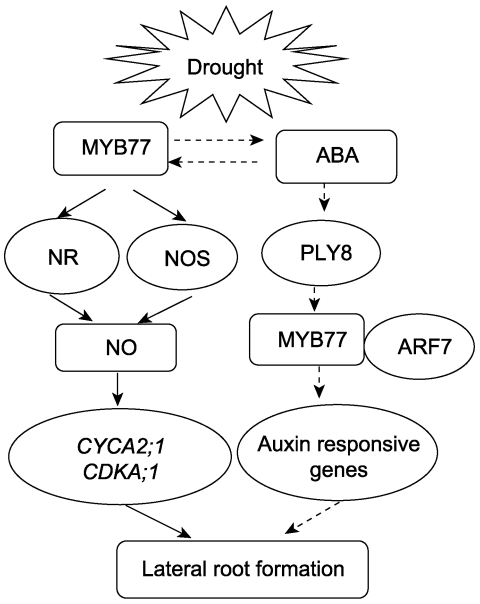

图6 AtMYB77参与干旱胁迫下拟南芥侧根发育的工作模型
实线部分为本文结果，虚线部分为根据已有报道及推测可能存在的作用。ABA: 脱落酸; NR: 硝酸还原酶; NOS: 一氧化氮合酶

Figure 6 Working model of AtMYB77 function in regulating lateral roots development under drought stress in *Arabidopsis*
The solid lines indicate the results of this study, and the dotted lines indicate the possible roles based on reports and speculation. ABA: Abscisic acid; NR; Nitrate reductase; NOS: Nitric oxide synthase

侧根起源于母根上特定的中柱鞘建成细胞，中柱鞘建成细胞感受刺激被激活，重新进行细胞分裂。细胞周期蛋白(cyclins，CYC)及其依赖性激酶(cyclin dependent kinases，CDK)在细胞的分裂与增殖中发挥重要调节作用。Correa-Aragunde等(2006)研究表明, NO影响细胞周期调控基因*CYCA2;1*、*CYCA3;1*、*CYCD3;1*、*CDKA1*和*KRP2*等的表达，进而调控侧根的形成。我们的研究结果也显示, *AtMYB77*过表达及NO供体或清除剂均能影响细胞周期核心调控因子基因*CYCA2;1*和*CDKA*;*1*的表达量，表明干旱胁迫下MYB77通过NO影响细胞周期调节基因的表达，进而调控侧根发育。*Atmyb77-1*缺失突变体中响应干旱诱导*CYCA2;1*和*CDKA*;*1*表达上调的作用减弱，但其表达量与野生型差异不显著; *AtMYB77*缺失或过表达显著影响干旱条件下侧根的生长，但对正常条件下侧根生长的作用不显著。推测这些现象可能是由于*AtMYB-77*与其它同源基因存在功能冗余或存在蛋白水平的调节等有关。例如, ABA受体PLY8可与MYB77直接作用促进侧根的发育，PLY8与MYB44和MYB73存在类似相互作用，因此MYB44和MYB73可能部分弥补了MYB77的作用(Zhao et al., 2014)。Lee等(2009)在研究甜椒(*Capsicum annuum* cv. ‘Pukang’) Rma1H1在干旱胁迫响应中的作用时发现类似现象, Rma1H1提高了转基因拟南芥的抗旱性，但是过表达株系中*Rma1H1*的转录水平与植株的存活率不完全一致，推测可能与存在蛋白水平的调控有关。

NO供体和清除剂不能完全抵消*AtMYB77*缺失或过表达对侧根发育的影响，推测MYB77除了通过NO调节侧根发育外还可能存在其它途径。生长素是调节侧根发育的主要植物激素。Shin等(2007)研究表明, MYB77可与生长素响应因子ARF7直接结合。Zhao等(2014)和Xing等(2016)研究发现，生长素响应基因*IAA19*等的启动子区域的生长素响应元件AuxREs与MYB转录因子结合元件紧密相邻，推测MYB77与ARF协同作用促进*IAA19*等生长素响应基因的表达，进而促进侧根的发生。ABA是主要的水分胁迫信号，参与侧根发育的调控，ABA受体PLY8和PLY9能与MYB77互作，增强MYB77与*IAA19*等生长素响应基因启动子的结合，促进其表达，进而促进侧根生长。干旱胁迫下，MYB77除了通过NO影响细胞周期，促进侧根发育，是否还与生长素信号途径有关联？干旱胁迫下MYB77对生长素信号途径的影响是ABA依赖还是非ABA依赖过程(图6)？这些问题的探究将有利于进一步完善干旱胁迫下侧根发育的调控机制。

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AtMYB77 Involves in Lateral Root Development via Regulating Nitric Oxide Biosynthesis under Drought Stress in *Arabidopsis thaliana*

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Abstract Both transcription factor MYB77 and signal molecule nitric oxide (NO) are important regulators of lateral root development. However, our understanding about the role of MYB77 and NO in the regulation of lateral root formation in plants remains elusive. This study investigated the roles and interrelation of MYB77 and NO in regulating lateral root formation under drought stress by using wild type *Arabidopsis*, AtMYB77 deletion mutant *Atmyb77-1* and overexpression lines AtOE77-1 and AtOE77-3. The results showed that the expression of AtMYB77 was induced by drought stress. When subjected to drought stress treatment, the *Atmyb77-1* mutant showed down-regulation of *CYCA2;1* and *CDKA;1*, two genes that are related with lateral root development. Meanwhile, the number and length of lateral roots in the *Atmyb77-1* mutant were significantly lower than those in wild type, while AtOE77-1 and AtOE77-3 lines displayed more and longer lateral roots. These results indicated that AtMYB77 was involved in the regulation of lateral root development under drought stress. We also showed that drought stress could increase the NO content, as well as the nitric oxide synthase (NOS) and nitrate reductase (NR) enzymes activity and gene expression in roots of *Arabidopsis*. Such increase in NO content, NOS and NR activities as well as related gene transcript levels were attenuated by deletion of AtMYB77 but enhanced by AtMYB77 overexpression. Exogenous NO donor sodium nitroprusside (SNP) alleviated the inhibitive effects of AtMYB77 deletion on the expressions of *CYCA2;1* and *CDKA;1* as well as the lateral root formation, while NO scavengers or synthesis inhibitors attenuate the promoting effect of AtMYB77 overexpression on lateral root growth. Taken together, these results demonstrate that AtMYB77 participates in drought-induced lateral root growth by promoting NO synthesis.

Key words AtMYB77, NO, lateral root development, drought stress, *Arabidopsis thaliana*

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